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REVIEW

What's new in the diagnosis of pancreatic cancer: a patent review (2011-present)

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ABSTRACT

Introduction: Pancreatic cancer (PC) is the fourth leading cause of cancer-related death in the US with a 5-year survival rate of about 5%. Most patients have advanced metastatic disease mainly due to the lack of an effective early detection, and an extremely poor prognosis. Advancing in the fight against PC requires developing novel observable biomarkers at preclinical stages for early detection.

Areas covered: This manuscript is an overview of different PC diagnostic modalities and the latest innovations made to enhance early PC detection through the patents published from 2011 to 2017. It also comments on the ongoing clinical trials and highlights the main challenges to be addressed in the future.

Expert opinion: At present, real efforts are being made to identify new specific biomarkers with a potential clinical applicability, and to develop new devices that integrate several biomarkers in order to be more sensitive and specific for the early detection of PC. Although many biomarkers have been patented recently, they will not reach the clinic until they have been validated by clinical trials. We believe that the high-throughput screening of '-omic' technologies to detect tumor-specific molecular alterations can lead to an enhanced understanding of the disease mechanisms and the discovery of new clinical diagnostic biomarkers.

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1. Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer-related death in the USA with a 5-year survival rate of about 8%. PC death rates have continued to increase slightly (by 0.3% per year) in men while they have leveled off in women. This is because the early stage of the disease is largely asymptomatic and the later symptoms are usually nonspecific and varied. Therefore, PC is often not detected until it is at an advanced stage, for which the 5-year survival rate is 3%. As a result, more than 85% of patients have tumors that cannot be resected at the time of diagnosis, with a high incidence of metastasis and a subsequent high mortality rate [1]. However, when PC tumors are less than 10 mm at the time of diagnosis, their resection permits achieving a 5-year survival rate of 75%. Moreover, patients with PC who were accidentally diagnosed for unrelated illnesses during imaging have a longer median survival time than patients who are symptomatic [2]. Thus, the likelihood of successful treatment and survival increase with early detection of PC. In the study carried out by Yachida et al., they have suggested that from the onset of the tumor until metastasis, around 21 years elapse [3]. This leaves a reasonable amount of time to allow for an early diagnosis. Due to that, screening of high-risk populations is a key issue. An effective screening program targeted at high-risk populations may help in the prevention and early detection of PC [4–6]. More than 5% of all pancreatic tumors are related to hereditary factors which include familial PC (FPC), Peutz-Jeghers syndrome, familial adenomatous polyposis (FAP), and familial atypical multiple mole melanoma (FAMMM). Other causes of PC risk are tobacco, diabetes mellitus, alcohol abuse, dietary factors, exposure to toxic substances, and chronic pancreatitis [7–9]. These factors have an impact on the development of PC when compared to healthy populations. All of these people are potential candidates for screening programs and are essential to help increase the performance of a putative screening test to make it cost-efficient.

Currently, a combination of different techniques, including tumor markers, imaging tests and biopsies, are used in the clinic to detect PC.

1.1. Serum tumor markers

Blood tests are usually used to help in the diagnosis or to determine treatment options. In a blood test, serum bilirubin and alkaline phosphatase levels can point to PC but they are not diagnostics [10]. The most common serum-specific tumor markers used are cancer antigen (CA) 19-9, CA15-3 and CA72-4 t [11], although CA 19-9 is the most widely used as it can help to confirm the diagnosis and predict prognosis and recurrence. However, it has a limited specificity and sensitivity and it cannot distinguish between pancreatitis and cancer or other disease states with a chronic process of inflammation [12]. In this context, a clinical trial is currently being developed aimed at studying whether CA 19-9 can be helpful in

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Article highlights

(1) The high rate of PC mortality is due to the lack of symptoms at the early stage of the disease and to the fact that the later symptoms are usually nonspecific and varied. Thus, it is necessary to determine new biomarkers that are present and observable at preclinical stages in ordere to be useful for early PC detection.
(2) Screening of high-risk populations is crucial in the prevention and early detection of PC.
(3) Neither CEA nor CA 19-9, the two tumor biomarkers most commonly used in the clinic for detecting PC, appear to be sensitive and specific enough to accurately diagnose PC early. Their main clinical application is as markers to monitor progression and response to treatment.
(4) High-throughput screening – 'omic' technologies that detect tumor-specific molecular alterations, including genomics, epigenetics, non-coding RNA, microbiome and metabolomics signatures, are revolutionizing this field.
(5) Liquid biopsy, including ctDNA, microRNAs and exosomes as a less invasive approach, seem to be the future of early PC detection, because of its remarkable advantages.

This box summarizes key points contained in the article.

monitoring recurrence and progressive disease in patients with PC [13]. Although this clinical trial is still in the recruitment phase, many of the patents focused on the search for new PC-specific biomarkers, registered in the last 2 years use CA 19-9 either as a target or as a reference marker [14–17]. Moreover, carcinoembryonic antigen (CEA), which is not used as often as CA, is a glycoprotein where rising levels are associated with adenocarcinoma, including colon cancer, breast cancer, and stomach cancer. The level of CEA has a significant correlation with tumor size, its differentiation and liver metastasis [18].

1.2. Imaging tests

Imaging techniques play a great role in PC detection. They include transabdominal ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET)-CT, endoscopic retrograde cholangiopancreatography (ERCP), endoscopic US (EUS), fine needle aspiration (FNA), somatostatin receptor scintigraphy (SRS) and angiography [18,19].

US are appropriate for the initial image, and is the first test performed due to its ease and innocuity, since it does not expose the patient to radiation. In addition, CT scans are often used to diagnose PC because they can show the pancreas fairly clearly and can be used to guide a biopsy needle into a suspected pancreatic tumor. Dual-phase helical CT scanning is the best option, if available, because it is the most sensitive test and it is not invasive in PCs and distant metastases [10]. When a tumor is not identified with the aforementioned imaging techniques, further MRI, or magnetic resonance cholangiopancreatography is indicated to test if the pancreatic and bile ducts are blocked, narrowed, or dilated [12]. EUS is more accurate than US and permits a hollow needle to be passed down the endoscope to obtain biopsy samples. It has a good ability to detect pancreatic masses, but it is not sufficient for the differential diagnosis of various types of lesions. In 2011, a patent was registered to improve this technique by developing a computer-aided method for distinguishing the EUS image of PC and extracting and classifying its textural features [20]. Furthermore, new techniques have been developed to improve the characterization of the lesions detected by EUS, including (i) EUS-guided FNA (EUS-FNA), developed to characterize lesions detected by EUS, which is particularly useful for diagnosing pancreatic tumors and assisting in making treatment decisions. It has a diagnostic sensitivity of 54–96%, a specificity of 96–98%, and an accuracy of 83–95% for PC [21]; (ii) EUS elastography (EUS-E) to measure tissue elasticity, color patterns, and tension ratio [21]; and (iii) contrast-enhanced endoscopic US (CE-EUS) which uses an ultrasonographic contrast agent to visualize blood flow in fine vessels. This technique is useful in the diagnosis of pancreatic solid lesions and in confirming the presence of vascularity in mural nodules for cystic lesions. Applications of CE-EUS in PC also incorporate and improve new methods such as perfusion imaging for monitoring ablative therapy or local detection of portal vein, or the use of the US to induce the liberation of drugs [22].

Another imaging technique in PC diagnosis is ERCP used only when other techniques are not conclusive and the probability for malignancy is high. Although its diagnostic ability seems to be limited in cases of extrinsic biliary strictures such as PC, recent studies have reported that brushing cytology and aspiration cytology using an endoscopic nasopancreatic catheter placed during ERCP improve the diagnostic accuracy in PCs [23].

ERCP is also appropriate when specialists consider relieving biliary obstruction [10]. When doctors use ERCP, they can collect the pancreatic juice and cells for pathological examination. However, it is invasive and might cause some related complications for the patient, such as perforation or pancreatitis [18].

Last, a PET scan is sometimes used to look for spread from exocrine PCs. PET-CT with uorine-18 uorodeoxyglucose is a combination of PET and high-end multi-detector-row CT, widely utilized in PC to evaluate the response to radiotherapy and to detect the metabolic activity in the pancreas [18]. Until now, routine screening for PC in high-risk population is not automatically recommended by the specialists, because there are still some unresolved problems in PC screening. Indeed, there is no ideal single screening method or screening program for detection of early PC [2], and their effectiveness in reducing mortality remains to be proven [4]. For this reason, efforts are aimed at improving the determination of tumor markers to aid in the diagnosis of presymptomatic PC, treatment assessment, and monitoring for disease recurrence. Below is a summary of patents and literature on different PC diagnostic modalities and the latest innovations made to enhance early PC detection through the patents published from 2011 to the present, by PubMed, Patentscope (https://patentscope.wipo.int/search/es/search.jsf), lens.org (https://www.lens.org/lens) and Espacenet (https://worldwide.espacenet.com/?locale=en_EP), using 'pancreatic cancer biomarkers,' 'early detection of pancreatic cancer,' 'liquid biopsy and pancreatic cancer,' and 'diagnostic pancreatic diagnostic' as key words.

2. What's new in diagnostics?

Due to all the limitations of current diagnostic techniques and the inability for early detection, enormous efforts are being made to improve the techniques. This is manifested through a large number of patent registrations in recent years reviewed, especially in 2016.

2.1. Specific biomarkers for PC

Currently, there have been no tumor markers that allow reliable screening at an early and potentially curative stage of the disease used in clinical settings. Hence, there is still a need to identify PC markers that are present and observable at preclinical stages that can be useful for early disease detection in order to improve PC mortality. In this context, new biomarkers of PC were identified in different body fluid samples (Figure 1) and several patents have been registered (Table 1).

2.1.1. Proteomic markers

2.1.1.1. Diagnostic biomarkers in tissue, serum, and bile.

In the last few years, many proteomic markers specific to PC have been discovered. In 2013, 170 proteins were selected and patented as PC markers for specific detection and prognosis [39]. In March 2016, a clinical trial was completed, the objective of which was to analyze serum proteins directly by mass spectrometry to identify new biomarkers of PC for early diagnosis [40]. Recently, in December 2016, 23 types of proteins or fragments thereof were patented as PC biomarkers [24]. These biomarkers (AP3B1, C2, CKS1B, CKS2, CSPG2, CYCS, CD82, PI3, RNASE1, RNASET2, VRK2, EV1, HMGB2, MST4, MMP9, MYBL2, PPM1B, AK3L1, IGFBP2, STMN1, ANXA6, ATP6AP1, and a protein or a fragment thereof selected from the group consisting of HYOU1) were identified by proteomic analysis using antibody libraries and plasma microarrays from body fluids (whole blood, serum, plasma, or urine) in more than 0.8 area under the curve at the time of performing the ROC analysis (AUC) values, making a distinction between healthy subjects and patients with invasive ductal carcinoma. This invention claims as a PC indicator the presence in the sample of (i) a high level of at least one of these biomarkers and/or (ii) a low level of HYOU1, compared to healthy controls. These biomarkers may be used to diagnose not only PC but also malignant tumors occurring in the pancreas and precancerous lesions and are capable of further improving the detection sensitivity of PC. Moreover, Del-1 protein-positive exosome was patented as a biomarker that notably increases in the blood of a cancer patient compared to that of a healthy person, and decreases in the blood of the patient after a surgery [25]. In particular, Del-1 protein-positive exosome enables diagnosis of a variety of cancers including PC. In addition, a dopamine receptor, in particular DRD2, was patented as a new biomarker for PC diagnosis [36]. Thus, through immunohistochemical and RT-PCR techniques, the detection of DRD2 in pancreatic tissues from patients (preferably from pancreatic ductal epithelium) was indicative of the presence of pancreatic tumor cells. The diagnostic method is preferably an *in vitro* or *ex vivo* method. In addition, a method was patented [16] for diagnosing PC, based on the detection of (i) at least one diagnostic amino acid being proline, histidine, or tryptophan, preferably proline, (ii) at least one diagnostic ceramide being ceramide (d18:1, C24:0) or ceramide (d18:2,C24:0), and (iii) at least one diagnostic sphingomyelin, being sphingomyelin (35:1), sphingomyelin (d17:1,C16:0), sphingomyelin (41:2) or sphingomyelin (d18:2,C17:0), preferably sphingomyelin (35:1). These biomarkers should always be compared to a reference marker such as CA 19-9. Sensitivity and specificity were adjusted so that the group of false negatives was minimal in order to efficiently exclude a subject for being at increased risk, or so that the group of false positives was minimal in order to efficiently assess a subject as being at an increased risk. High sensitivity (>85%) and high specificity (74.7–87.3%) were found to distinguish PC from chronic pancreatitis or non-pancreatic disease. Moreover, inventors included samples from 878 patients with

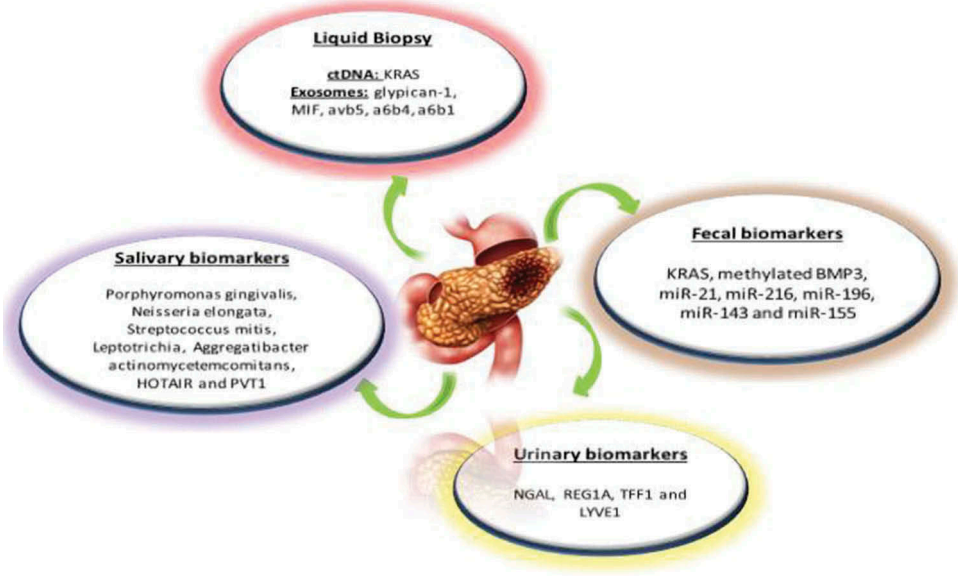

Figure 1. Novel biomarkers of PC in different body fluid samples.

Table 1. Recent patents related to PC biomarkers.

Biomarker	Sample	Patent Number	Year	Ref
CRP, ICAM-1, OPG, and CA 19-9	Human serum/plasma	WO2017008388 (A1)	**2017**	[14]
CRP, ICAM-1, OPG, and CA 19-9	Human serum/plasma	WO2017008389 (A1)	**2017**	[15]
Praline/histidine/tryptophan and ceramide (d18:1, C24:0/d18:2, C24:0), and sphingomyelin (35:1/d17:1, C16:0/41:2/d18:2, C17:0), and CA19-9.	Blood, plasma, serum, or urine	WO2016207391 (A1)	**2016**	[16]
4BPA, PIGR	Serum	US20150104816 (Al)	**2015**	[17]
AP3B1, C2, CKS1B, CKS2, CSPG2, CYCS, CD82, PI3, RNASE1, RNASET2, VRK2, EV1, HMGB2, MST4, MMP9, MYBL2, PPM1B, AK3L1, IGFBP2, STMN1, ANXA6, ATP6AP1, HYOU1	Blood, serum, plasma, or urine	WO2016195051 (A1)	**2016**	[24]
Del-1 protein-positive exosome	Blood	WO2016148313 (A1)	**2016**	[25]
LYVE1, REG1, and TFF1.	Urine, whole blood, serum, or pancreatic tissue	WO2016124947 (A1))	**2016**	[26]
ERBB2, ESR1, and TNC	Biopsy, biological fluid	WO2016049045 (A1)	**2016**	[27]
Platelet Glycoprotein V (GP5)	Blood, plasma, or serum sample.	WO2016030426 (A1)	**2016**	[28]
S100A1 1, M-CSF, C3adesArg, CD26, IL-8, CEA, VEGF, and CRP	Pancreatic tissue	WO2016001249 (A1)	**2016**	[29]
Metabolomic signature	Plasma, blood, or serum	WO2016097860 (A1)	**2016**	[30]
miR-21, miR-23a, miR-23b, and miR-29c	Salivary samples	WO2016113233 (A1)	**2016**	[31]
ANO1, C19orf33, EIF4E2, FAM108C1, IL1B, ITGA2, KLF5, LAMB3, MLPH, MMP11, MSLN, SFN, SOX4, TMPRSS4, TRIM29 and TSPAN1.	Pancreatic tissue	US2016055297 (A1)	2016	[32]
hsa-miR-27a-5p, hsa-miR-183-5p, and hsa-miR-425-5p.	Blood Pancreatic tissue			
hsa-let-7g-3p, hsa-miR-7-2-3p, hsa-miR-23a-5p, hsa-miR-27a-5p, hsa-miR-92a-1-5p, hsa-miR-92a-2-5p, hsa-miR-122-5p, hsa-miR-154-3p, hsa-miR-183-5p, hsa-miR-204-5p, hsa-miR-208b-3p, hsa-miR-425-5p, hsa-miR-510-5p, hsa-miR-520 a-5p, hsa-miR-552-3p, hsa-miR-553, hsa-miR-557, hsa-miR-608, hsa-miR-611, hsa-miR-612, hsa-miR-671-5p, hsa-miR-1200, hsa-miR-1275, hsa-miR-1276, and hsa-miR-1287-5p				
IL-11 (interleukin)	Plasma or serum	CN104698170 (A) KR20150030046 (A)	2015 2015	[33] [34]
PALB2, BRCA2, pl6, PMS2, MLH1, MSH2, STK11, MSH6, or EPCAM gene	Blood, serum, plasma, urine, fecal, buffy coat, buccal swabs, saliva or a pancreatic tissue	WO2015157557	2015	[35]
Dopamine Receptors (DRD2)	Pancreatic tissues (preferably from pancreatic ductal epithelium)	WO2015158890 (A2)	2015	[36]
Antibodies and fragments thereof (chimeric, murine, humanized or human PAM4 antibodies)	Serum	CA2899811 (A1) US20140112864 (A1)	2014	[37] [38]

other diseases/comorbidities such as prostate cancer, lung cancer, diabetes, or hypertension, among others. The classification score showed a remarkably high disease specificity of the biomarker panels of the invention, also with regard to the panels in comparison to the CA19-9 as a single marker. Other proteins such as platelet glycoprotein V [28] were patented to determine a subject's probability of suffering from PC, and ERBB2, ESR1, and TNC [27] biomarkers, with high specificity to binding to early stage PCs.

Moreover, in 2016 various studies were reported on the highly specific and sensitive biomarkers found in bile. A lipocalin family member, glycoprotein neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, has emerged, playing an important role in the development, progress, and invasion of cancer. The concentration of this protein in bile (sensitivity: 71.43% and specificity: 80%) and urine (sensitivity: 80.95% and specificity: 80%) has been found to be remarkably accurate in differentiating chronic pancreatitis from PC [41]. Furthermore, a soluble LDL receptor relative with 11 ligand-binding repeats (sLR11), a molecule released from immature cells, was evaluated in 147 samples of bile from patients with biliary tract cancer, PC, or benign diseases. Results showed that bile sLR11 levels in cancer patients were significantly higher than in those without cancer, while bile CA 19-9 and CEA levels were not different [42].

2.1.1.2. Antibodies and vaccines. Recently, much emphasis has been placed on exploring the signature of another important component of serum proteins: antibodies raised against autoantigens of the tumor, such as autoantibodies. Although the detailed molecular mechanism that causes and generates autoantibodies against specific tumor antigens is still unclear, it has been suggested that antibodies are formed against mutated, misfolded, overexpressed, aberrantly degraded proteins or differently glycosylated glycoproteins. Several studies have described weak antibody-mediated immune responses against tumor antigens in PC. Thus, antibodies against Rad1, calreticulin isoforms and acidic isoforms of enolase (i.e. ENOA1/2) were observed in 7, 28, and 62% of PC patients, respectively. Moreover, anti-MUC1 IgG antibodies correlated strongly with survival time ($p = 0.0004$) [43]. Patents related to the anti-MUC1 IgG have been registered [37,44]. These patents describe an organic solvent extraction of serum method before immunoassay to detect anti-PC antibodies or fragments thereof (murine, chimeric, humanized, or human PAM4 antibodies). The antibodies showed novel and useful diagnostic characteristics, such as binding to a high percentage of early stage PCs, but not to normal or benign pancreatic tissues. Antibodies bind preferentially to PC mucins such as MUC or MUC5ac, and are applied at the early detection stage. The authors suggested performing an immunoassay with anti-CA19.9 and PAM4 antibodies to increase PC sensitivity. In fact, the results showed a PC detection sensitivity of 77.4%, with a detection specificity of 94.3%, comparing pancreatic carcinoma ($n = 53$) with all other specimens ($n = 233$), including pancreatitis and

breast, ovarian and colorectal cancer and lymphoma. The ROC curve provided an AUC of 0.88, a highly significant difference for distinguishing PC from non-pancreatic carcinoma samples. The immunohistology procedure employing the PAM4 antibody identified approximately 90% of invasive PC and its precursor lesions [37].

Furthermore, a human monophosphorylated alpha-enolase isoform was patented [45], containing a single phosphorylation on the serine residue at position 419 of the human alpha-enolase amino acid sequence, as well as antibodies capable of specifically binding the peptide and/or the isoform of the invention. The peptide, isoform and antibodies of the invention are useful in the diagnosis and/or treatment of pancreatic adenocarcinoma. Moreover, another invention claims that detection of anti-ezrin autoantibodies in an '*in vitro*' assay can be used as a biomarker for early detection of pancreatic ductal adenocarcinoma in patients or persons predisposed to pancreatic ductal adenocarcinoma [46].

Furthermore, PSMC5, TFRC, and PPP1R12A are also shown to be targets of a clinically relevant antibody response induced with a vaccination. They are strongly overexpressed in PC whereas they are either weakly or not expressed at all in pancreatic normal duct cells. Thus, they were patented as biomarkers that increase during pancreatic tumor development and have demonstrated a favorable disease-free survival rate [47].

2.1.2. Genetic markers

Numerous patents have focused on the identification of genes and gene combinations that are correlated with patients that have or are predisposed to developing PC. In this context, there is a patent on a method for diagnosing pancreatic ductal adenocarcinoma (PDAC) based on the determination, in a biological sample, of the expression level of at least two genes from this panel: epithelial cell transforming sequence 2 oncogene (ECT2); AHNAK nucleoprotein 2 (AHNAK2); serpin peptidase inhibitor, Glade B (ovalbumin) member 5 (SERPINB5); transmembrane protease, serine 4 (TMPRSS4); periostin, osteoblast specific factor (POSTN); S100 calcium binding protein P (S100P); carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5); gamma-am inobutyric acid (GABA) A receptor, pi (GABRP); chymotrypsin-like elastase family, member 2B (CELA2B); and CUB and zona pellucid-like domains 1 (CUZD1) [48]. The 5-gene PDAC classifier predicted PDAC with high accuracy in 9 independent validation sets with a sensitivity value of 96% and specificity of 85.7%, and an AUC of 0.9 in validation sets that contained PDAC and normal pancreas samples, which is significantly better than CA19-9, and exhibited a specificity of 85.7 and 86.67%, respectively. Moreover, the classifier distinguished between PDAC and benign pancreatic disorders, with a specificity of 88.9% and sensitivity 100%, an overall accuracy of 93.3% and AUC of 0.94 [48]. Another patent through a panel of genes provides methods for classifying a subject diagnosed with PDAC as having an activated stroma subtype or a normal stroma subtype of PDAC and/or a basal subtype or a classical subtype of PDAC [49]. Moreover, mutated PALB2 gene [35], and BRCA2, pl6, PMS2, MLH1, MSH2, STK11, MSH6, or EPCAM genes [50] were patented as a method for screening in blood, serum, plasma, urine, feces, buffy coat, buccal swabs, saliva, or a pancreatic tissue sample of patients with PC.

2.1.3. Metabolite markers

In the cancer research field, metabolomics studies can lead to an enhanced understanding of disease mechanisms and to the discovery of new diagnostic biomarkers. Currently, it is known that numerous metabolites define a metabolomic signature for distinguishing malignant from benign tissues. There are several studies relating to some metabolites including palmitic acid, 1,5-Anhydo-D-glucitol, combined xylitol, 1,5-anhydro-D-glucitol, histidine, and inositol as potential biomarkers for PC [51]. However, there are hardly any related patents registered. The patent WO2016097860A1 [30] describes a method of diagnosing and classifying PC by examining the expression of particular metabolites that distinguish this disease state from benign disease and periampullary adenocarcinoma. The predominant differences are within carbohydrate and amino acid metabolic pathways. The research method is based on the determination, by Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance in PC plasma, blood, or serum samples, of high levels of at least four biomarkers of the following: isopropanol, galactose, mannose, arabitol, trimethylamine-noxide, threitol, succinate, tridecanol, trehalose-alpha, azelaic acid, isoleucine, tyrosine, pyroglutamate, arginine, creatine, lysine, and alanine. The efficiency level of this method is similar to the serum tumor marker CA 19-9, implying that the metabolic characterization of pancreatic tumor has clinical benefits. Also, metabolites such as Tridecanol, Match, Pyroglutamate, azelaic acid, isoleucine, arginine, tyrosine, alanine, lysine, and creatine are elevated in benign pancreatic lesions.

Moreover, other metabolite markers containing C4b-binding protein alpha chain (C4BPA) or polymeric immunoglobulin receptor (PIGR), which distinguished between chronic pancreatitis or normal subject and PC in pool serum samples collected before and after the operation, were patented. The AUC was 0.843 for CA19-9, 0.859 for C4BPA and 0.728 for PIGR. Also, when CA19-9, C4BPA, and PIGR were combined, the AUC was 0.939 [17]. These results indicate that although they are not specific for the diagnosis of PC, they can be used in combination with another PC marker such as CA 19-9, for monitoring postoperative prognosis.

2.1.4. MicroRNAs markers

Several microRNAs have also been patented as biomarkers for PC. In this context, a high level of hsa-miR-135b associated with an alteration of the level of at least one additional miR gene product (selected from hsa-miR-024, hsa-miR-096-P, hsa-miR-148a, hsa-miR-155, hsa-miR-196a, hsa-miR-210, hsa-miR-217, hsa-miR-223, and hsa-miR-375) in the biological sample compared to a control, is indicative that the subject either has or is at risk for developing PC [52]. According to the patent CN 103861121 (A) [53], the miR491-5p presents a differential expression between the normal pancreas and the PC tissue, so it can be used as the PC diagnosis marker. In addition, microRNA expression pattern has proved to be a valuable prognostic tool for predicting the survival of patients

diagnosed with PC as described in the patent [54]. Moreover, many efforts were made to identify PC-specific miRNAs in saliva. Thus, 94 miRNAs determined in saliva and blood samples from PC patents were selected from the literature search. From the group of miR-21, miR-23a, miR-23b, and miR-29c, at least one miRNA expression level was determined. The difference between baseline values and salivary samples, in miRNA expression level, is a diagnostic tool for predicting a patient's PC risk and can also be used to monitor treatment. The patent [31] also includes a description of kits (primers, probes, microarrays or macroarrays) that involve a measurement system for the miRNA expression level. Currently, a clinical trial is underway to recruit patients at different stages of PC disease in order to test their blood, bile and tissue samples to search for lipidomics, proteomics, microRNAs, and volatile organic compound biomarkers [55].

2.2. Sensors and other devices for PC detection

Applications in nanomedicine such as diagnostics and targeted therapeutics rely on the detection and targeting of membrane biomarkers. In this context, the patent US2012100560A1 [56] utilizes quantitative profiling, spatial mapping, and multiplexing of cancer biomarkers such as EpCAM, MUC1, MUC3, MUC4, MUC16, and CEA, by functionalized quantum dots. This approach provides highly selective targeting molecular markers for PC with extremely low levels of nonspecific binding, and provides quantitative spatial information of biomarker distribution in a single cell, which is important since tumor cell populations are inherently heterogeneous. Another invention was patented in relation to the application of a PC immunosensor, based on gold electrodeposition and Au@Ag/CuO-GS, as a marker for detecting PC. By using the excellent biocompatibility and high catalytic performance of the Au@Ag/CuO-GS, the prepared sensor has relatively high sensitivity and a relatively broad detection range, and the detection limit can reach 1.5 fg/mL [57]. Moreover, a platinum hybrid copper oxide multiwalled carbon nanotube immunosensor was patented to detect common PC tumor markers [58]. This sensor has important scientific significance and application value in PC detection due to it high specificity, high sensitivity, and low detection limit. In the technical field of novel functional materials and biological sensing detection, a patent was recently registered on a CA19-9 – Pt-carbon nitride/graphene tumor marker sensor [59]. A Pt nanoparticle has a good catalytic yield to hydrogen peroxide. Therefore, the large surface area of carbon nitride/graphene is used to fixedly charge the Pt nanoparticle to act as a marker for detecting an antibody, enabling the super flexible detection of the tumor marker CA19-9. Hence, this sensor is of great importance for the early diagnosis and prognosis of the tumor marker. Furthermore, a diagnostic kit based on the detection of the expression of DRD2 in pancreatic tissue was patented as a diagnostic method for detecting chronic pancreatitis or pancreatic tumor [36]. Other diagnostic kits use a combination of ABAT (4-aminobutyrate aminotransferase) and CA19-9 proteins, or CHI3L1 (Chitinase-3-like protein 1 precursor) and CA19-9 proteins as biomarkers. These protein chip kits are useful for predicting or diagnosing in a blood sample the onset, the possibility of onset, and the severity of PC in an early stage, and were applied in a study on the tumorigenesis of PC [60]. Recently, a detection chip for the PC protein biomarkers CRP, ICAM-1, OPG, and CA 19-9 in the human serum/plasma was patented [14,15]. This chip is based on an antigen-antibody specificity combination and provides increased accuracy and specificity.

In addition, there are also devices that detect genes and microRNAs as described in the patent [32]. This invention is based on the detection of genes specifically expressed in PC patients (ANO1, C19orf33, EIF4E2, FAM108C1, IL1B, ITGA2, KLF5, LAMB3, MLPH, MMP11, MSLN, SFN, SOX4, TMPRSS4, TRIM29, and TSPAN1) or a panel of microRNAs obtained from blood or tissues paired with the genes. Results in 84 PC patients and 84 healthy persons showed a sensitivity to detect PC of 83% and a specificity of 81% [32]. Recently, in June 2016, a clinical trial was initiated aimed at developing integrated analytical methods of genomic data and clinical data and analyzing the biological control network. This will allow acquiring knowledge based on the integrated analysis system with the subsequent determination of biomarkers for early diagnosis and treatment of PC and ultimately a customized disease management system. The trial will also confirm the effectiveness of a diagnostic chip for research purposes by applying the pancreatic/bile duct cancer-specific biomarker miRNA, found through the integrated analysis of genomic data and clinical data of patients with pancreatic/bile duct cancer, to the blood of patients with pancreatic/bile duct cancer [61].

3. Conclusion

In summary, 37 patents for new biomarkers in PC diagnosis have been revised as shown in Table 2. The scientific community is making real efforts to identify new specific biomarkers with a potential clinical applicability for PC and its early detection. Many patents have focused on the expression profiles of various molecular markers in different stages of PC, which

Table 2. Patents revised for new diagnostic of pancreatic cancer.

Year	# Patents	Reference
Proteomic Markers		
2016	10	[16,24–29,40,44,47]
2015	1	[33]
2014	2	[37,38]
2013	2	[39,46]
2011	2	[34,45]
Genetic Markers		
2016	2	[48,49]
2015	1	[35]
2012	1	[50]
Metabolomic Markers		
2016	1	[30]
2015	1	[17]
microRNAs Markers		
2016	1	[31]
2014	3	[52–54]
Sensors and other devices for PC detection		
2017	2	[14,15]
2016	2	[32,60]
2015	3	[36,57,59]
2014	1	[58]
2012	1	[56]
2011	1	[20]

could reveal the role of these molecules in the progression of PC and their potential value to detect previous disease. However, the diagnostic value of the results obtained is uncertain because not enough clinical trials have been carried out and when they have been performed, the number of subjects with early PC lesions included have not been enough to ensure the robustness and reliability of the studies to allow routine clinical application Multicenter trials must be implemented and there must be meta-analysis in large cohorts to corroborate the data obtained in patents and to establish a panel of biomarkers that will be useful in the early detection of PC. Moreover, these novel biomarkers are far from being widely distributable and affordable for healthcare systems because they must be cost effective and inexpensive. Accuracy in diagnosis must be improved to avoid false positive or negative results. Also, another disadvantage is that some of the techniques used for detecting novel PC biomarkers are not yet part of laboratories' clinical diagnosis. Therefore, new devices with higher sensitivity and specificity need to be developed, and more efforts must be made to simplify the technology used for its detection. Otherwise, it will be very complicated to apply them to clinical routines in the near future.

4. Expert opinion

Most patients with PC have advanced metastatic disease mainly due to the lack of an effective modality for early detection, resulting in an extremely poor prognosis. Currently, the detection and diagnosis of PC depends largely on expensive imaging modalities that are unable, even if combined, to detect PC and very small metastases early. Thus, there is an urgent need to find less invasive and more effective alternative methods to identify the disease early and predict progression in order to improve tumor monitoring. CEA and CA 19-9 are the most widely used blood-based tumor biomarkers for PC. For awhile, they appeared to be a good alternative, however, none of these tumor markers is sensitive and specific enough to accurately diagnose PC early, since their levels are not elevated in all PC patients. Therefore, their main clinical application is as markers for monitoring progression and response to therapy [2]. Hence, new effective biomarkers are required to improve early diagnosis, disease surveillance, and therapeutic choice for PC.

After reviewing patents registered in recent years, it is evident that there is increased attention to the detection of tumor-specific molecular alterations, including genomics, epigenomics, noncoding RNA, and metabolomics signatures, by high-throughput screening – '-omic' technologies. Figure 1 shows novel patented biomarkers of PC in different body fluid samples that might be relevant in the future. In this context, numerous novel driver mutations, such as gene expression changes, epigenetic alterations, chromosomal rearrangements, and copy number aberrations of PC, have been detected [51]. Thus, many new genes mutated in PCs, such as ATM, ARID1A, ROBO2, MLL3, TGFBR2, NOP14, and TUFT1 [62], have been identified as passenger mutations, since they are less common, but they could play an important role together with the most prevalent mutated genes such as KRAS.

Furthermore, epigenetic markers, including alterations in promoters, microRNA expression, and chromatin structure, might be used to improve early diagnosis of PC. Many studies in this field have provided a large number of new valuable and promising markers for the early detection of PC. Some patents in the manuscript provide early detection [27,28,44]. Obstacles which hinder the movement of biomarkers into clinical trial are shown in detail in reviews [63–65]. Usual biases and mistakes should be avoided if methodologies and guidelines are strictly followed, such as the retrospective blinded evaluation (PRoBE) design, prospective specimen collection [66], and the Standards for Reporting of Diagnostic Accuracy (STARD) statement [67]. To date, some biomarkers have been superior to CA 19-9 in sensitivity and specificity. Especially, oncogenic miRNA profiling, the subject of several patents [16,31,32] seems to be the most promising biomarker due to its stability in tissues and blood plasma. Moreover, it is readily detected by RT-PCR using suitable normalizers for each normal or PC sample, to ensure the robustness and reliability of the results in personalized medicine for PC [68].

Along with multiple genetic, epigenetic, and growth signaling alterations, cancer cells reprogram several metabolic pathways. Therefore, in the cancer research field, metabolomics and microbiome studies may lead to an enhanced understanding of disease mechanisms and to the discovery of new diagnostic biomarkers. In this context, several metabolites showed a better discriminating ability than CA 19-9 for distinguishing patients with PC from healthy controls [69,70]. A joint evaluation of these pre-therapeutic tumor markers to CA 19-9 would be interesting in order to significantly reduce the probability of detecting false positives and to improve the prognostic prediction in patients with PC. In addition, it is postulated that microbiota influences the susceptibility to PC through several pathways, which include nutrition, metabolism, hormonal homeostasis and inflammation. Some studies claim that the presence of some oral and gut bacteria increase the risk of developing PC. However, further studies are needed to elucidate their applicability as biomarkers of early PC diagnosis.

Currently, liquid biopsy, as a less invasive approach, is attracting much attention because of its remarkable advantages. The broad conception of the liquid biopsy comprises all tumor elements circulating in the blood, including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating microRNAs, exosomes, etc. [71]. In this context, PC can be considered as an accumulative process of various genetic aberrations, and the mutated genes in the bloodstream will provide a clue to the carcinogenesis of PC. Therefore, the less invasive and actionable ctDNA has great potential for pancreatic tumor screening amongst a high-risk population. Moreover, ctDNA may be a promising stable biomarker due to the existence of a positive correlation between ctDNA levels and tumor burden, and their levels are less influenced by intratumor heterogeneity than a single specimen of tumor tissue. Several studies have achieved a remarkable detection rate of ctDNA in patients with localized

PC, and demonstrate that the proportion of patients with detectable ctDNA increased according to the clinical stage. This point out that, despite the poor detection in early stage cases, the presence of mutations has high positive predictive value. However, other studies have been unable to detect any mutations in all patients. This shows low sensitivity due to the fact that amounts of nonmutated DNA might mask some vestiges of the mutant type of KRAS [51]. This is the same problem as that of CTCs. Although CTCs could be detected in the peripheral blood at the early stage of tumor formation, even before tumor formation, their use for early detection and diagnosis of PC remains unclear due to their low sensitivity. Furthermore, exosomes are small vesicular structures that carry various pathogenic miRNAs, mRNAs, DNA fragments, and proteins which play an important role in PC progression and can be used for the early detection of PC (Figure 1). However, they are present in the blood in a very small amount, which makes their isolation difficult and make the purification process a challenge. Although patents related to the use of liquid biopsy for the early diagnosis of PC are scarce, we have no doubt that many more will be registered in the coming years. Currently, there are two ongoing clinical trials that began at the end of last year. The first is focused on determining the correlation between the presence of CTCs and numbers of GPC1-exosome concentration, and clinical and biological parameters and patient clinical outcome [72]. The second is focused on the concordance between specific DNA mutations found in patient biopsies and plasma circulating tumor DNA [73]. The results of these two clinical trials will undoubtedly allow taking advantage of liquid biopsy as support for clinical decisions.

There is still a long, but very promising, road ahead before these new biomarkers are transferred to the clinical routine of patients with PC. We believe that the significant efforts made by researchers to fine-tune and simplify 'omic' techniques will enhance the effectiveness and reliability in the prediction and early diagnosis of this disease.

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Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Siegel R, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
2. Poruk KE, Firpo MA, Adler DG, et al. Screening for pancreatic cancer: why, how, and who? Ann Surg. 2013;257(1):17–26.
3. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010;467:1114–1117.
4. Overbeek KA, Cahen DL, Canto MI, et al. Surveillance for neoplasia in the pancreas. Best Pract Res Clin Gastroenterol. 2016;30(6):971–986.
•• **An effective screening program targeted at high-risk people, may help in prevention and early detection of PC.**
5. Canto MI, Hruban RH, Fishman EK, et al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology. 2012;142(4):796–804.
6. Kimmey MB, Bronner MP, Byrd DR, et al. Screening and surveillance for hereditary pancreatic cancer. Gastrointest Endosc. 2002;56:S82–S86.
7. Langner C. [Hereditary gastric and pancreatic cancer]. Pathologe. 2017;38(3):164–169.
8. Midha S, Chawla S, Garg PK. Modifiable and non-modifiable risk factors for pancreatic cancer: a review. Cancer Lett. 2016;381:269–277.
9. Urayama S. Pancreatic cancer early detection: expanding higher-risk group with clinical and metabolomics parameters. World J Gastroenterol. 2015;21:1707–1717.
10. Freelove R, Walling AD. Pancreatic cancer: diagnosis and management. Am Fam Physician. 2006;73(3):485–492.
11. Zhang X-P, Yu Z-X, Zhao Y-P, et al. Current perspectives on pancreatic serous cystic neoplasms: diagnosis, management and beyond. World J Gastrointest Surg. 2016;8(3):202–211.
12. De la Cruz MS, Young AP, Ruffin MT. Diagnosis and management of pancreatic cancer. Am Fam Physician. 2014;89(8):626–632.
•• **CA 19-9 has a limited specificity and sensitivity and it cannot distinguish between pancreatitis and cancer or surely other disease states with a chronic process of inflammation.**
13. Dickson D. A prospective longitudinal study of CA 19-9 as an aid in monitoring disease in patients with pancreatic cancer. ClinicalTrials.gov Identifier: NCT02250638. 2015.
•• **Clinical trial aimed to study if CA 19-9 can be an aid in monitoring recurrence and progressive disease in patients with pancreatic cancer.**
14. Zhang G, Pronyuk K, Kuliesh O, et al. Pancreatic protein biomarker, uses of same, detection chip therefor, and detection device. WO2017008388A1. 2017.
• **The invention features a detection chip for the PC protein biomarkers CRP, ICAM-1, OPG, and CA 19-9 in the human serum/plasma.**
15. Zhang G, Pronyuk K, Kuliesh O, et al. Detection reagent kit and detection system for pancreatic cancer protein biomarkers. WO2017008389A1. 2017.
16. Kamlage B, Reszka R, Schatz P, et al. Means and methods for diagnosing Pc in a subject based on biomarker panel. WO2016207391A1. 2016.
17. Nitto Boseki Co., Ltd. Marker for detecting pancreatic cancer. US 20150104816A1. 2015.
18. Zhang Q, Zeng L, Chen Y, et al. Pancreatic cancer epidemiology, detection, and management. Gastroenterol Res Pract. 2016;2016:1–10.
19. Poley JW, Kluijt I, Gouma DJ, et al. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. Am J Gastroenterol. 2009;104:2175–2181.
20. Zhang M, Jin Z, Li Z, et al. Computer-aided method for distinguishing ultrasound endoscope image of pancreatic cancer. CN102122356A. 2011.
•• **This invention relates a computer-aided method for distinguishing the ultrasound endoscope image of pancreatic cancer and extracting and classifying their textural features.**

21. Kamata K, Kitano M, Omoto S, et al. New endoscopic ultrasonography techniques for pancreaticobiliary diseases. Ultrasonography. 2016;35(3):169–179.
22. Saftoiu A, Vilmann P, Bhutani MS. The role of contrast-enhanced endoscopic ultrasound in pancreatic adenocarcinoma. Endosc Ultrasound. 2016;5(6):368–372.
23. Kamisawa T, Wood LD, Itoi T, et al. Pancreatic cancer. Lancet. 2016;388(10039):73–85.
24. Nat Cancer Center. Plasma biomarker panel for diagnosing pancreatic cancer. WO2016195051A1. 2016.
25. Exosome Co Ltd. Cancer diagnostic or prognostic biomarker composition comprising Del-1 protein positive exosome. WO2016148313A1. 2016.
    - **This invention provides Del-1 protein positive exosome as a biomarker that notably increases in the blood of a cancer patient compared to healthy person and decreases in the blood of the patient after surgery.**
26. Queen Mary University of London. Biomarkers for pancreatic cancer. WO2016124947A1. 2016.
27. Hutchinson Fred Cancer Res. Pancreatic cancer diagnostic. WO2016049045A1. 2016.
28. Roland A, Daniel A. Method for determining a subject's probability to suffer from pancreatic cancer. WO2016030426A1. 2016.
29. University of Lubeck, Randox Laboratories Ltd. Combinaisons de biomarqueurs pour utilisation dans le depistage du cancer du pancreas. WO2016001249A1. 2016.
30. Oliver F; BATHE. Uti Limited Partnership. Metabolomics for diagnosing pancreatic cancer. WO2016097860A1. 2016.
31. Cordelier P; INSERM. Methods for the diagnosis of pancreatic cancer. WO2016113233A1. 2016.
32. Lg Electronics Inc, UnivYonseilacf. Method for extracting biomarker for diagnosing pancreatic cancer, computing device therefor, biomarker for diagnosing pancreatic cancer and device for diagnosing pancreatic cancer including the same. US2016055297A1. 2016.
    - **This invention is based on the detection of genes specifically expressed in PC patients or a panel of microRNAs obtained from blood or tissues paired with the genes.**
33. Zhualin R. Method of taking IL-11 (interleukin) as biomarker in diagnosis and prognostic evaluation of pancreatic cancer. CN104698170A. 2015.
34. Yongseo University. Composition for pancreatic cancer diagnosis comprising complement factor I-specific binding polypeptide or antibody. KR20150030046A. 2011.
35. Ballard KL, Mcdade RL, Spain MD, et al. Methods of diagnosing pancreatic cancer and methods related thereto. WO2015157557A1. 2015.
36. Pouria J, Hoheisel J, Riazalhosseini Y. Pancreatic cancer therapy and diagnosis. WO2015158890A2. 2015.
37. Liu D, Gold DV, Chang CH, et al.; Immunomedics, Inc. Anti-mucin antibodies for early detection and treament of pancreatic cancer. CA2899811A1. 2014.
38. Gold DV, Goldenberg DM; Immunomedics, Inc. Anti-mucin antibodies for early detection and treatment of pancreatic cancer. US20140112864A1. 2014.
    - **This patent provides anti-mucin antibodies as biomarker for PC early detection.**
39. Univ Tohoku. Pancreatic cancer biomarker. JP2013027387A. 2013.
40. Fazlul S. Michigan center for pancreatic cancer molecular diagnosis: the serum protein microarray project. ClinicalTrials.gov Identifier: NCT00897494. 2016.
41. Hogendorf P, Durczyński A, Skulimowski A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) concentration in urine is superior to CA19-9 and Ca 125 in differentiation of pancreatic mass: preliminary report. Cancer Biomark. 2016;16(4):537–543.
42. Kensuke T, Meizi J, Wataru T, et al. Levels of soluble LR11/SorLA are highly increased in the bile of patients with biliary tract and pancreatic cancers. Clin Chim Acta. 2016;457:130–136.
43. Kaur S, Baine MJ, Jain M, et al. Early diagnosis of pancreatic cancer: challenges and new developments. Biomark Med. 2012;6(5):597–612.
44. Liu D, Gold DV, Chang CH, et al.; Immunomedics Inc. Anti-mucin antibodies for early detection and treatment of pancreatic cancer. US2016090413A1. 2016.
45. Novelli F, Tomaino B, Cappello P. An isolated monophosphorylated peptide derived from human alpha-enolase useful for diagnosis and treatment of pancreatic adenocarcinoma, antibodies directed against the said monophosphorylated peptide, and uses thereof. WO 2011030302 A1. 2011.
46. Novelli F, Cappello P, Capello M. A method and a kit for the *in vitro* diagnosis of pancreatic ductal adenocarcinoma or for determining the predisposition to pancreatic ductal adenocarcinoma. WO 2013186748 A1. 2013.
47. Jaffee EA, Jhaveri DT, Anders R; Univ Johns Hopkins. Diagnostic biomarkers and therapeutic targets for pancreatic cancer. US2016327560A1. 2016.
    - **This invention provides PSMC5, TFRC, and PPP1R12A proteins as biomarkers that increase during pancreatic tumor development.**
48. Khosavifar R, Libermann TA, Bhasin MK; Beth Israel Deaconess Medical Center Inc. Methods and kits for the diagnosis and treatment of pancreatic cancer. US2016348182A1. 2016.
49. Moffitt R, Yeh JJ, Rashid NU; Univ North Carolina. Methods and compositions for prognostic and/or diagnostic subtyping of pancreatic cancer. WO2016061252A1. 2016.
50. Vogelstein B, Kinzler KW, Parsons DW, et al.; The Johns Hopkins University. Diagnostic method using palb2. US20160032406A1. 2012.
51. Zhou B, Xu JW, Cheng YG, et al. Early detection of pancreatic cancer: where are we now and where are we going? Int J Cancer. 2017;15;141(2):231–241.
52. Schmittgen TD, Brackett DJ; Univ Oklahoma, Ohio State Innovation Foundation. MicroRNA expression profile associated with pancreatic cancer. US2014357702A1. 2014.
    - **The invention includes a method of diagnosing and classifying pancreatic cancer by examining the expression of particular metabolites that distinguish this disease state from benign disease and periampullary adenocarcinoma.**
53. Li L, Ring G; Inst Basic Med Sciences Pla. Use of micro-RNA molecule miR491-5p in treatment and/or diagnosis and/or prognosis of pancreatic cancer. CN103861121A. 2014.
    - **The invention describes that high level of hsa-miR-135b associated with alteration of the level of at least additional miR gene product are indicative that the subject either has or is at risk for developing PC.**
54. Johansen JS, Schultz NA, Wojdemann M; Herlev Hospital. Microrna biomarkers for prognosis of patients with pancreatic cancer. US20140106985A1. 2014.
    - **This patent shows that miR491-5p presents differential expression between the normal pancreas and the PC tissue, so that it can be used as the PC diagnosis marker.**
55. Uday Navaneethan MD. Lipidomics, proteomics, micro RNAs and volatile organic compounds biomarkers in bile and serum in the diagnosis of malignant biliary strictures. ClinicalTrials.gov Identifier: NCT02531607. 2016.
56. Searson PC, Lee KH, Konstantopoulos K, et al.; The Johns Hopkins University. Device for capture, enumeration, and profiling of circulating tumor cells. US2012100560A1. 2012.
57. Univ Jinan. Preparation method and application of PC immunosensor based on gold electrodeposition and Au@Ag/CuO-GS as markers. CN104459132A. 2015.
58. Yueyun L, Liping J, Jian H, et al.; Univ Shandong Technology. Preparation method and application of platinum hybrid copper oxide multi-walled carbon nanotube sensor. CN104181299A. 2014.
    - **This patent provides a high specific, sensitive, and low detection limit sensor to detection on common PC tumor markers.**
59. Univ Jinan. Preparation method and application of Pt-carbon nitride/graphene carbon nitride tumor marker CA19-9 biological sensor. CN104880559A. 2015.
    - **The invention provides a sensor with importance for the early diagnosis and prognosis of the tumor marker.**

60. Han S; Sk Telecom Co Ltd, Seoul Nat UnivR&Db Found. Composition for diagnosing pc and method for diagnosing pancreatic cancer using same. US2016252512A1. 2016.
61. Si Young Song MD; LG Electronics Inc. Validation of useful markers generated by next generation bio-data based genome research and cohort study (miRNA_Chip). ClinicalTrials.gov Identifier: NCT02807896. 2016.
•• **Development of integrated analytical methods of genomic data, clinical data, and analysis of the biological control network. This will allow the acquisition of knowledge based on the integrated analysis system with the subsequent determination of biomarkers for the early diagnosis and treatment of pancreatic cancer.**
62. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518:495–501.
63. Kulasingam V, Diamandis EP. Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. Nat Clin Pract. 2008;5:588–599.
64. Diamandis EP. Cancer biomarkers: can we turn recent failures into success? J Natl Cancer Inst. 2010;102:1462–1467.
65. Pavlou MP, Diamandis EP, Blasutig IM. The long journey of cancer biomarkers from the bench to the clinic. Clin Chem. 2013;59:147–157.
66. Pepe MS, Feng Z, Janes H, et al. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. J Natl Cancer Inst. 2008;100:1432–1438.
67. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Clin Chem. 2003;49:7–18.
68. Morata-Tarifa C, Picon-Ruiz M, Griñan-Lison C, et al. Validation of suitable normalizers for miR expression patterns analysis covering tumour heterogeneity. Sci Rep. 2017;7:39782.
•• **This study underlines the importance of select suitable normalizers to cover tumor heterogeneity and to ensure the robustness and reliability of qPCR data for analyzing miR expression.**
69. Di Gangi IM, Mazza T, Fontana A, et al. Metabolomic profile in pancreatic cancer patients: a consensus-based approach to identify highly discriminating metabolites. Oncotarget. 2016;7:5815–5829.
70. Sakai A, Suzuki M, Kobayashi T, et al. Pancreatic cancer screening using a multiplatform human serum metabolomics system. Biomark Med. 2016;10:577–586.
71. Gao Y, Zhu Y, Yuan Z. Circulating tumor cells and circulating tumor DNA provide new insights into pancreatic cancer. Int J Med Sci. 2016;13(12):902–913.
72. Buscail E; University Hospital, Bordeaux. Diagnostic accuracy of circulating tumor cells (CTCs) and onco-exosome quantification in the diagnosis of pancreatic cancer – PANC-CTC. ClinicalTrials.gov Identifier: NCT03032913. 2017.
•• **Determination of the correlation between CTCs presence and numbers of GPC1-exosome concentration, with clinical and biological parameters and with patient clinical outcome.**
73. Kelsen J; Aarhus University Hospital. Circulating tumor cells and tumor DNA in HCC and NET. ClinicalTrials.gov Identifier: NCT02973204. 2016.
•• **Clinical trials focused on the concordance between specific DNA mutations found in patient biopsies and plasma circulating tumor DNA.**